The distribution of sugars and amino acids between source and sink organs: more than just a transporters' game

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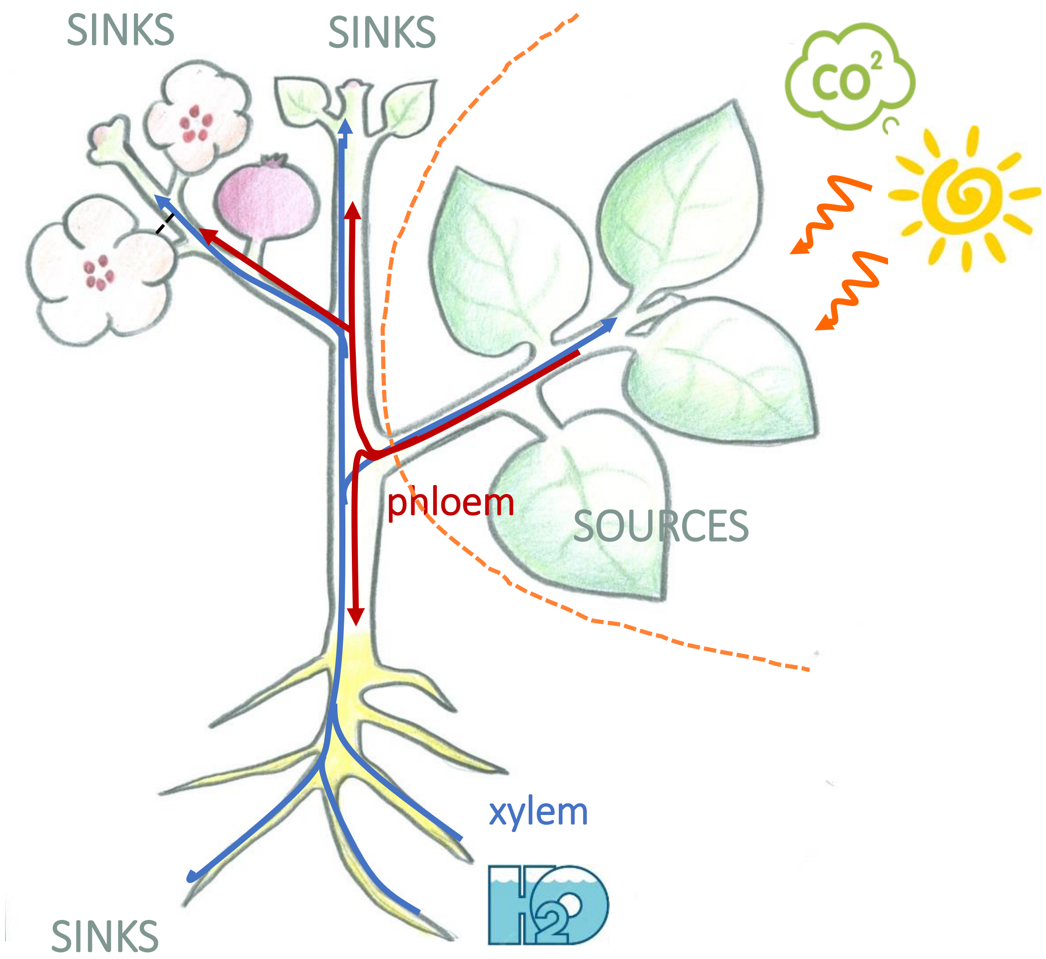
Source and sink organs for carbon allocation

Phloem transport: photoassimilates i.e. sugars, amino acids, organic acids, ions, signals, macromolecules



CARBON SOURCEs

Autotrophic organs
Mature leaves are net sources of carbon

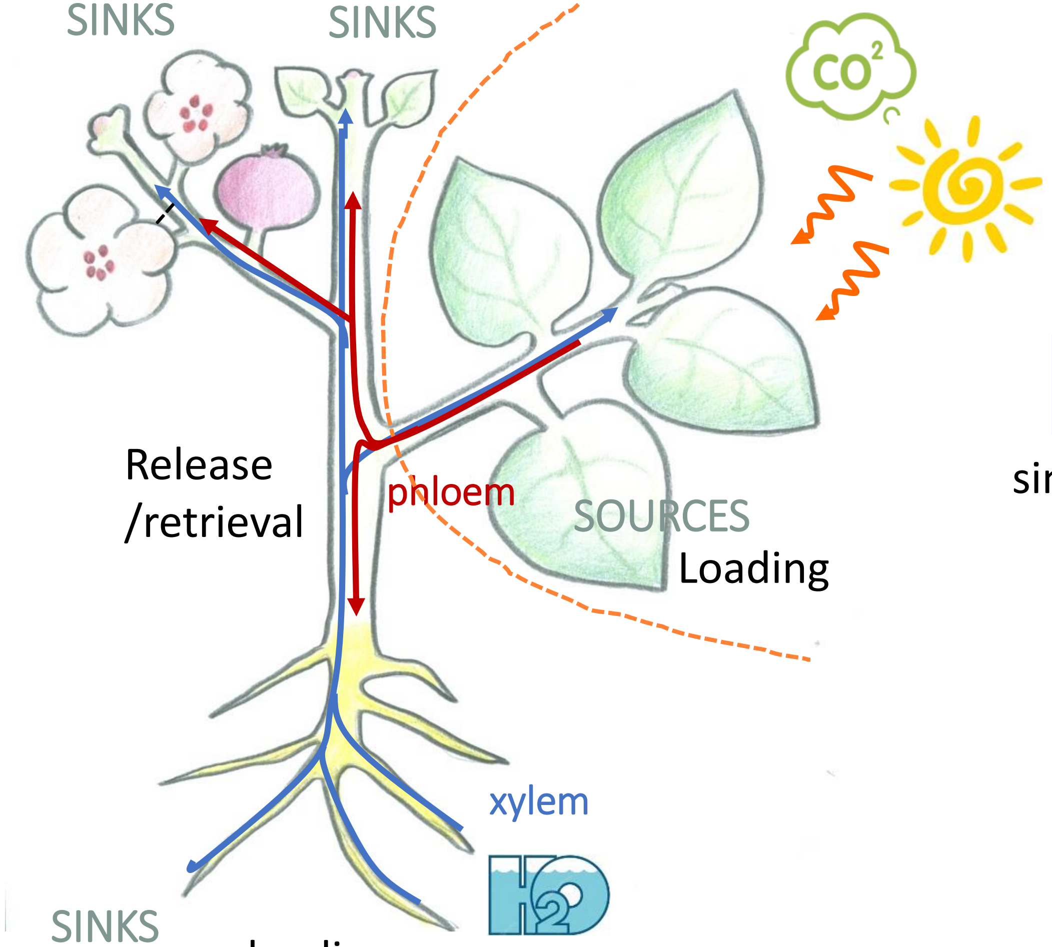
CARBON SINKs

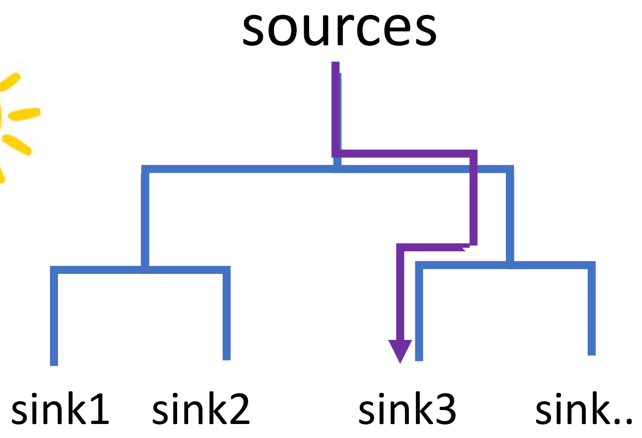
Heterotrophic tissues and organs
Net carbon sink tissues include roots, tubers, reproductive structures, young leaves, buds and meristems

MAIN FORM OF C TRANSPORT

Sucrose is the predominant sugar transported by the phloem
Other sugars (raffinose family of oligosaccharides, polyols)

Source and sink organs for carbon allocation

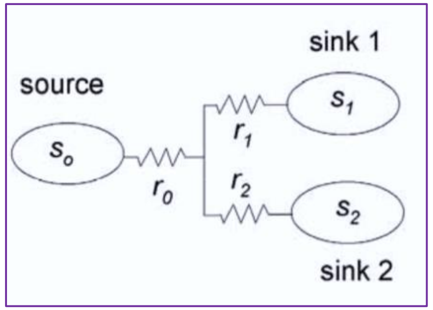




Source-to-sink relations

SOURCE strength

SINK strength

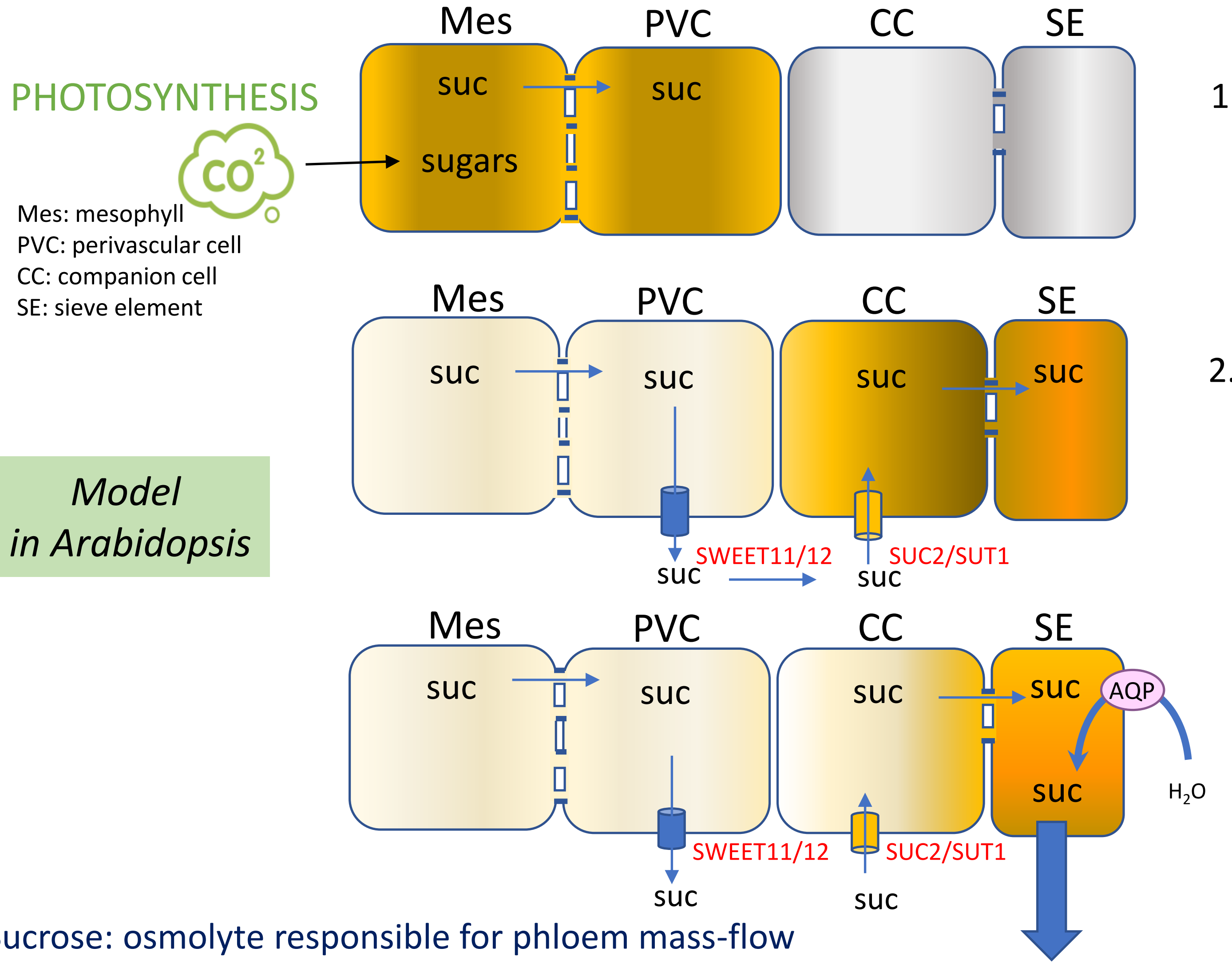


Coupling with water influx (from the xylem)

Modeling phloem and xylem transport

Modeling phloem transport, Minchin, Hölttä, Holbrook, Jensen, Lacointe, Durand, Gaudillère, Bohr....

Sucrose is central for phloem loading



Mes: mesophyll
PVC: perivascular cell
CC: companion cell
SE: sieve element

Model in Arabidopsis

1. Carbon fixation and production of sucrose (high osmolarity)

2. Efflux in the apoplasm by SWEET facilitators
   Influx in the cytosol by SUC/H+ symporter
   -> high concentration in CC-SE

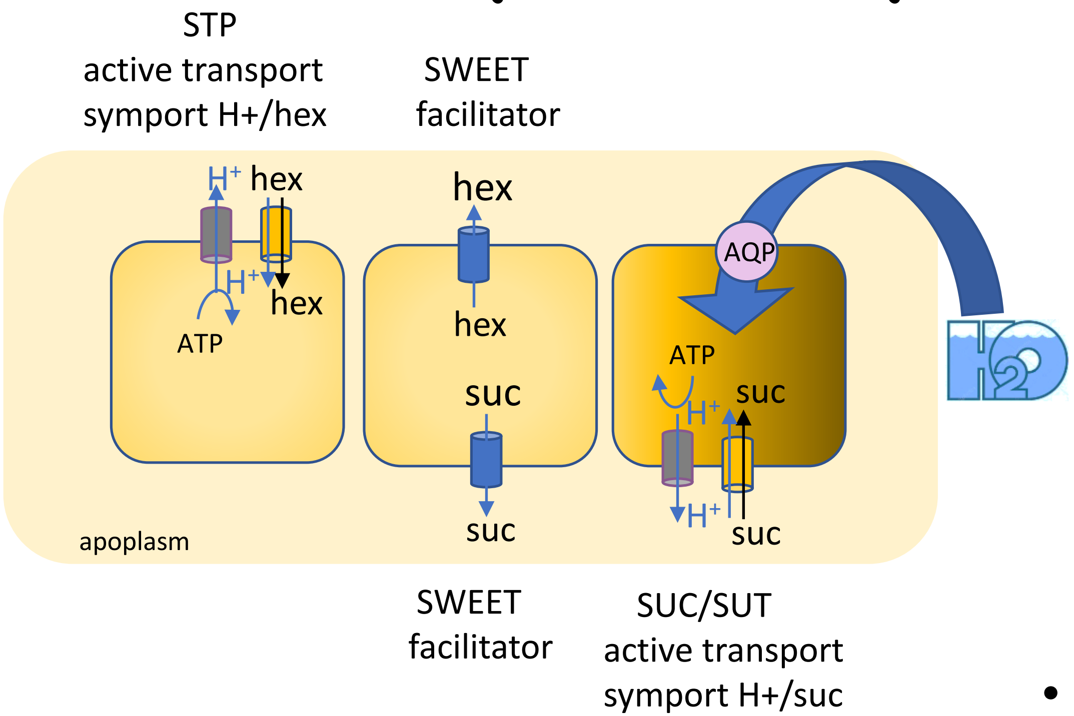
   sugar facilitators SWEET11 &SWEET12
   sucrose/H^+ symporter SUC2/SUT1

3. Entry of water by osmosis
   Propels bulk flow -> Mass flow
   - sugars
   - others molecules

Sucrose: osmolyte responsible for phloem mass-flow

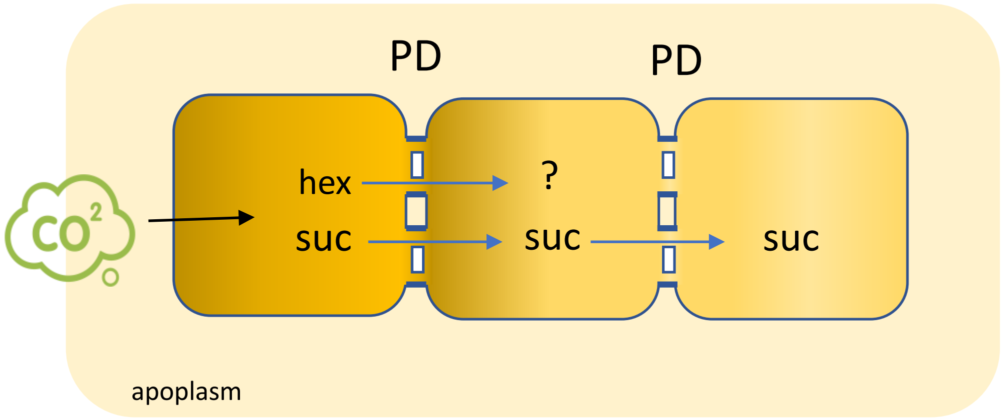
Apoplasmic versus symplasmic pathways

APOPLASMIC PATHWAY



A diversity of mechanisms for loading depending on species

SYMPLASMIC PATHWAY



- How do PDs and sugar transporters sugar 'talk' to each other?
- How do PD regulate sugar diffusion?
- how the level of sugars in the apoplasm is regulated?

Sucrose is not the only osmolyte generating the osmotic pressure in the sieve elements

pressure-flow mechanism: loading of osmolytes, principally sugars, into the phloem, generate the osmotic pressure that propels bulk flow

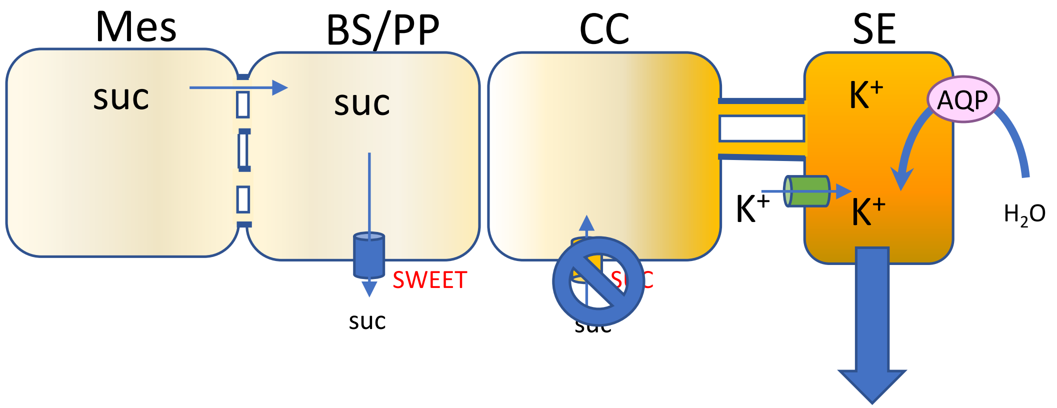
→ *loading of ions such as K+ into the phloem may compensate when sucrose loading is extremely low*

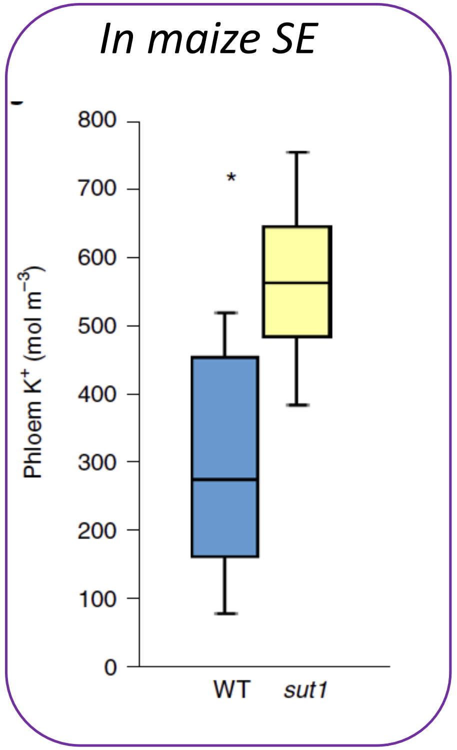
nature plants 2022 ARTICLES
https://doi.org/10.1038/s41477-022-01098-x
Check for updates

Sugar loading is not required for phloem sap flow in maize plants

Benjamin A. Babst[1,8], David M. Braun[2], Abhijit A. Karve[1,9], R. Frank Baker[2], Thu M. Tran[2], Douglas J. Kenny[1,10], Julia Rohlhill[1,11], Jan Knoblauch[3], Michael Knoblauch[3], Gertrud Lohaus[4], Ryan Tappero[5], Sönke Scherzer[6], Rainer Hedrich[6] and Kaare H. Jensen[7]

Mes: mesophyll
BS: bundle sheat
PP: phloem parenchyma
CC: companion cell
SE: sieve element

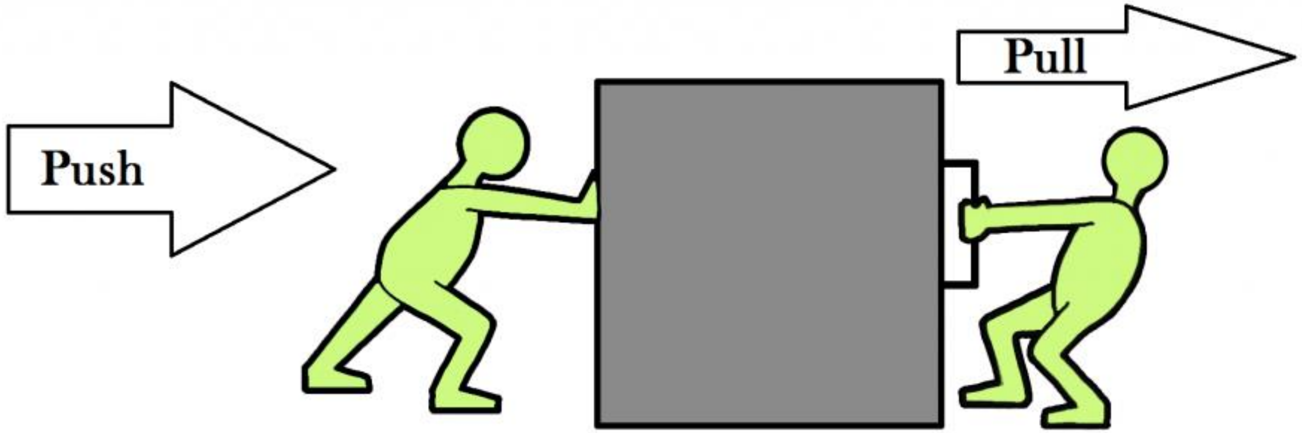




X-ray fluorescence (XRF) microscopy

Source-to-sink relations

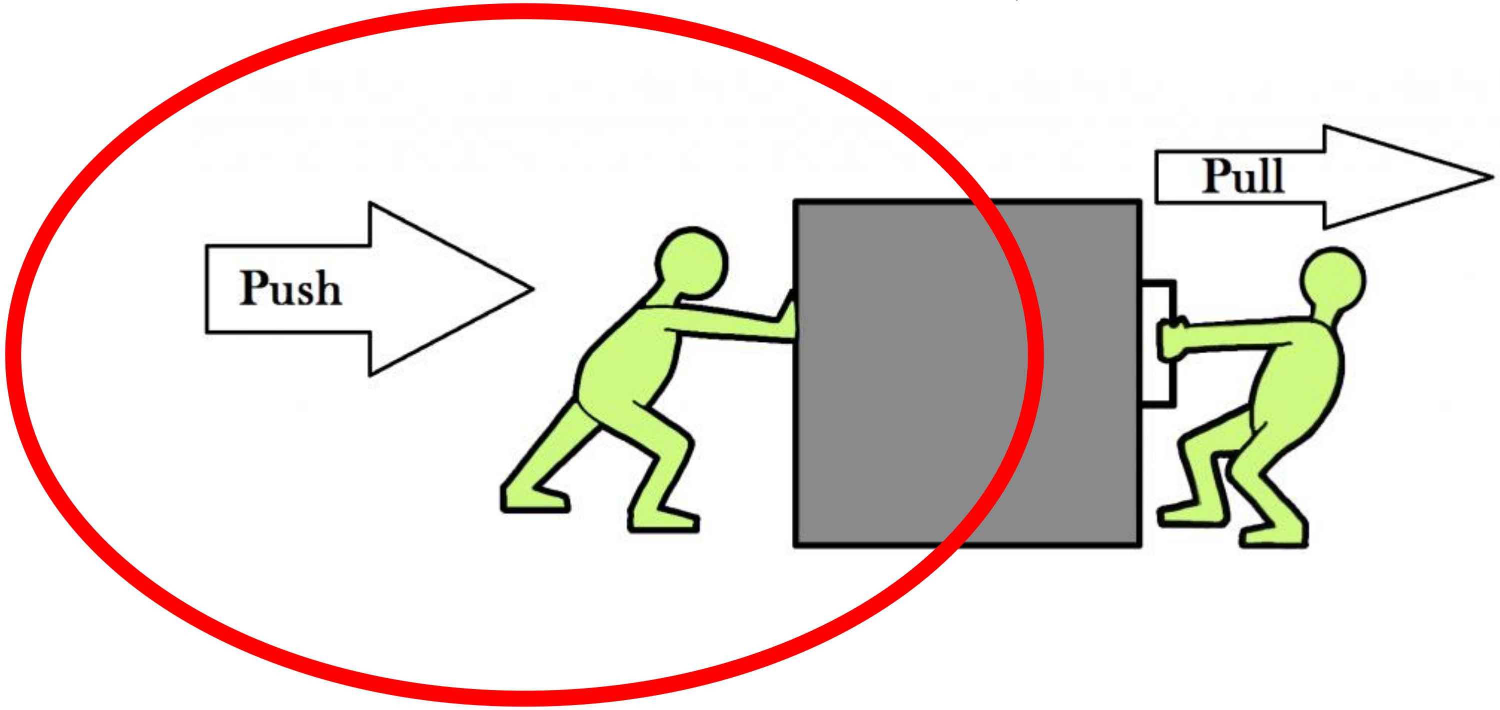
‘ Push or pull ? ‘



Source strength or sink strength?

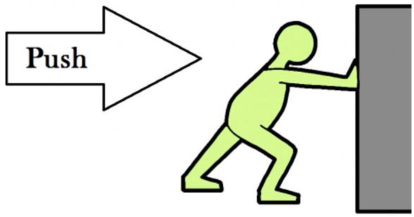
Source-to-sink relations

‘ Push or pull ? ‘



Source strength or sink strength?

Phloem loading and source strength
1) turn over of sugar transporters



Effect of environment on phloem transport (dark, low light, high light, drought)

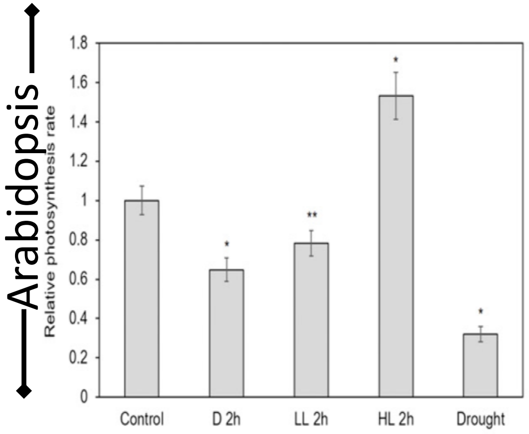
→ Sugar loading by active transporters SUC/SUT

-> Variations in phloem export in different conditions

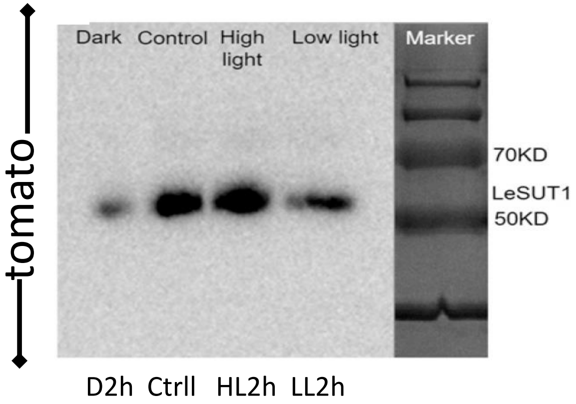
-> associated with SUC2/SUT1 protein levels

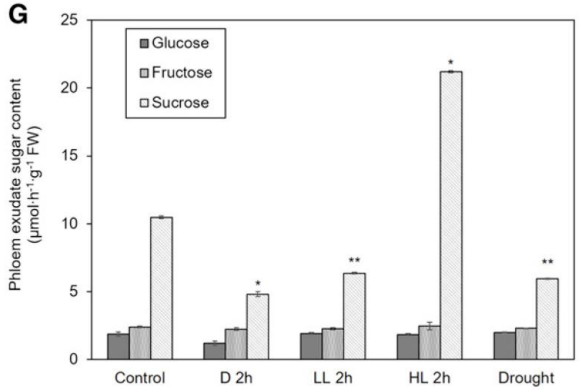
-> SUC2 is regulated by ubiquitination & phosphorylation (Xu et al PNAS 2020)

Photosynthesis → Protein accumulation → Phloem export (Exudation rate)

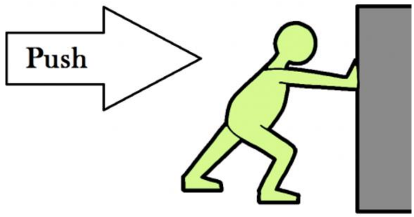


Xu et al 2018 Plant phys





Phloem loading and source strength
2) vein anatomy

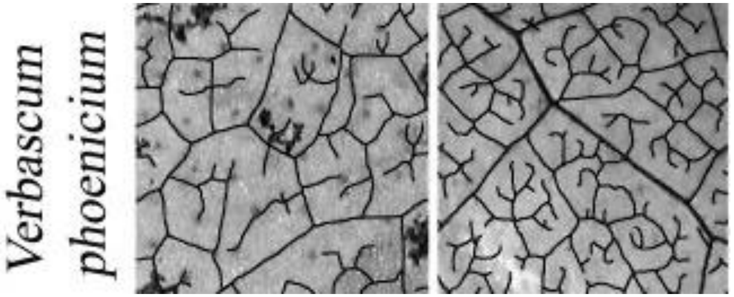


→ Sugar loading occurs in the minor veins

→ Plasticity in the vein density
- density and complexity of minor veins

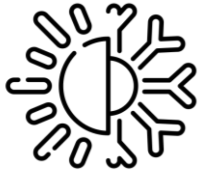
Vein density (symplasmic loaders)

Low light (LL) High light (HL)

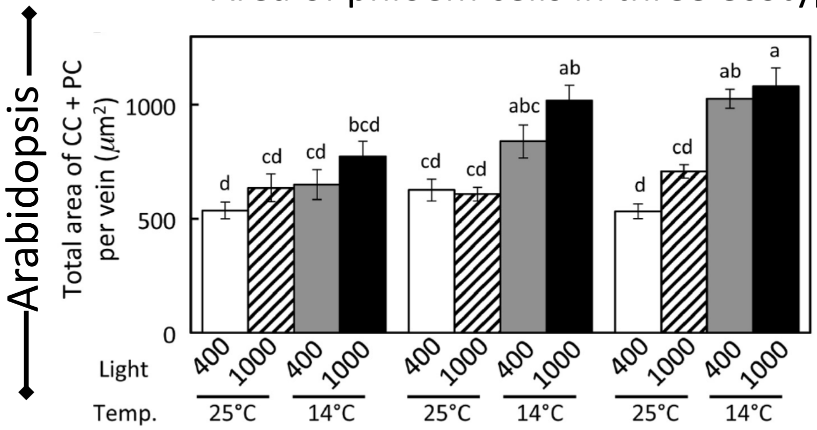


Amiard et al 2005 PNAS

→ Plasticity in the anatomy of the phloem
- nb of cells, size of cells
- invagination of PM in transfer cells

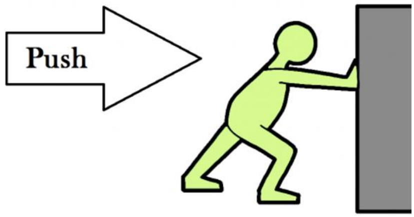


Area of phloem cells in three ecotypes

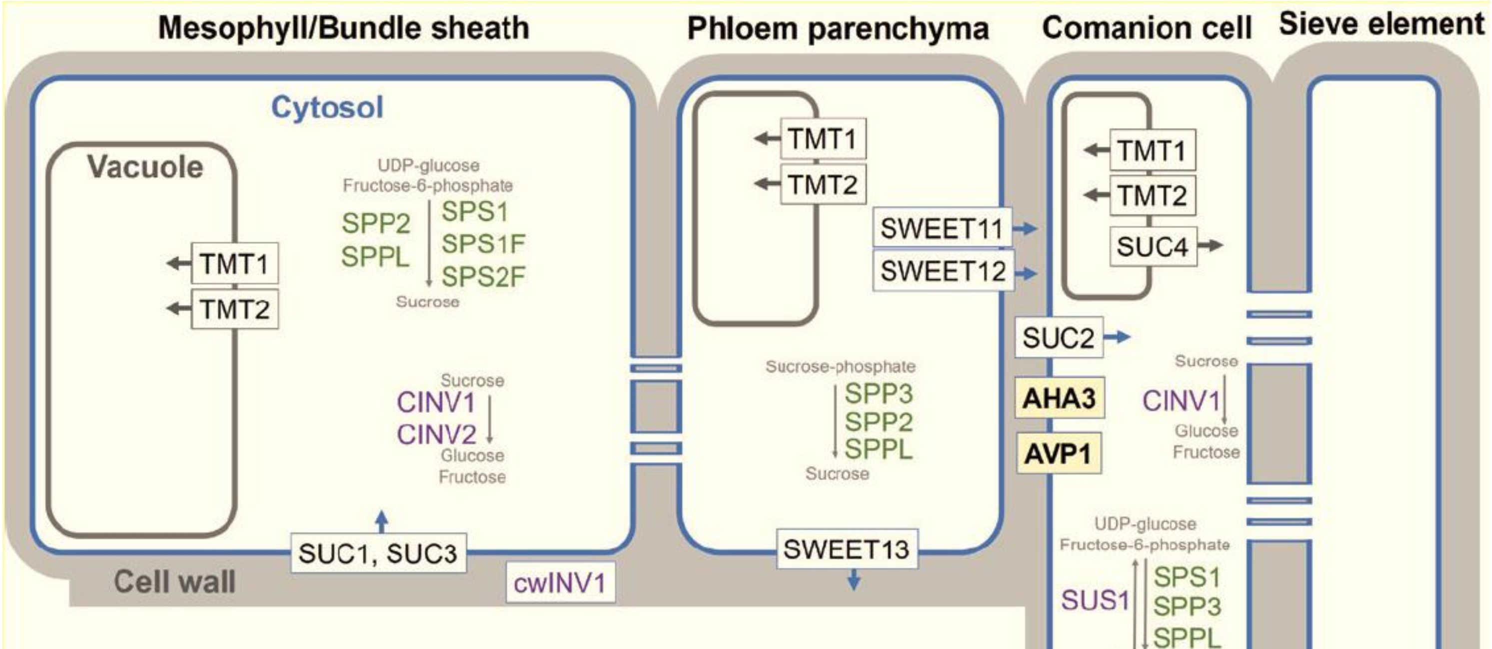


Cohu et al 2013 FIPS

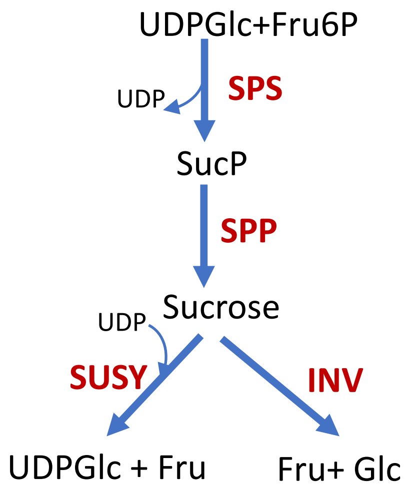
Phloem loading and source strength
3) many sugar transporters



Model
in Arabidopsis



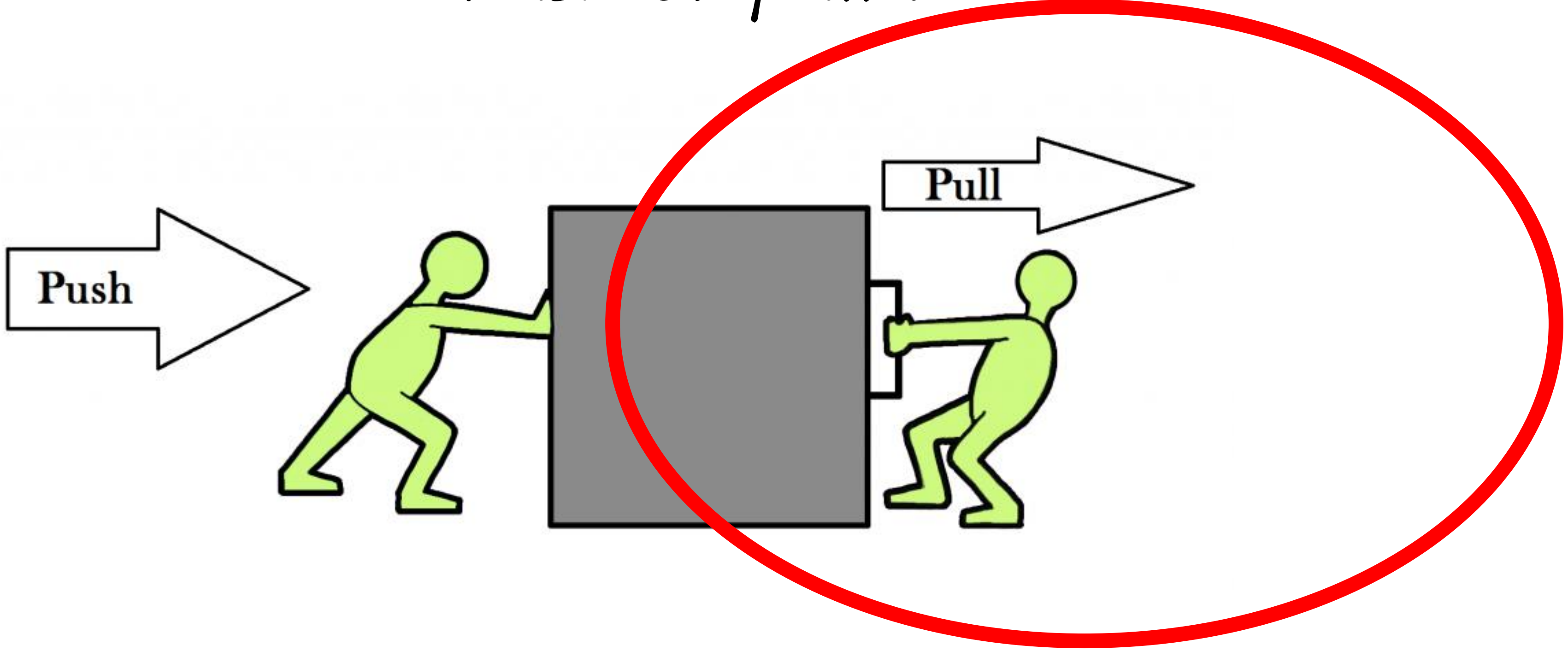
Xu & Liesche 2021 JXB, extract from the transcriptome data
based on transcriptomic data from Kim et al 2021 Plant Cell



→ A range of sugar transporters - sucrose/hexose families
→ A range of enzymes acting on sucrose availability (INVERTASE, SUSY, SPP, SPS)
→ No information on the selectivity/activity of plasmodesmata

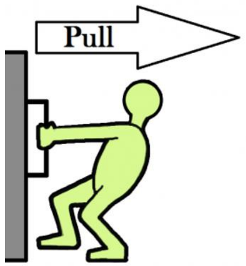
Source-to-sink relations

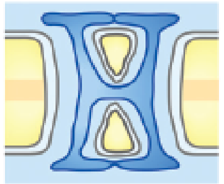
' Push or pull ? '



Source strength or sink strength?

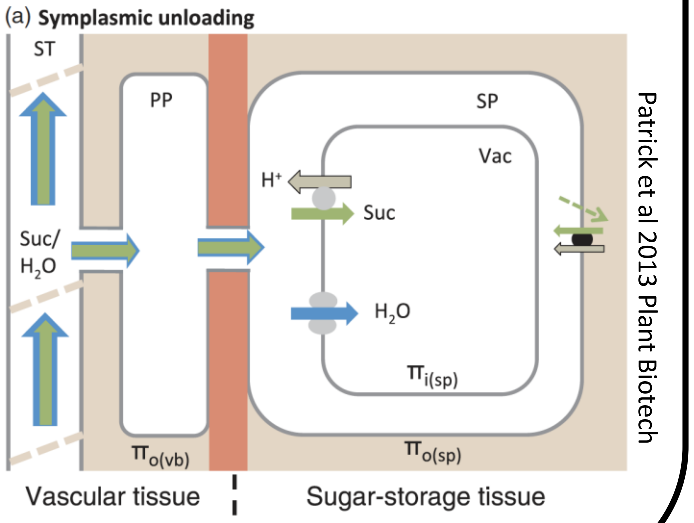
Phloem unloading and sink strength
1) symplasmic or apoplasmic ?



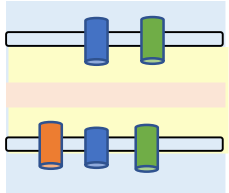


Specialized PD
Simple, branched or funnel

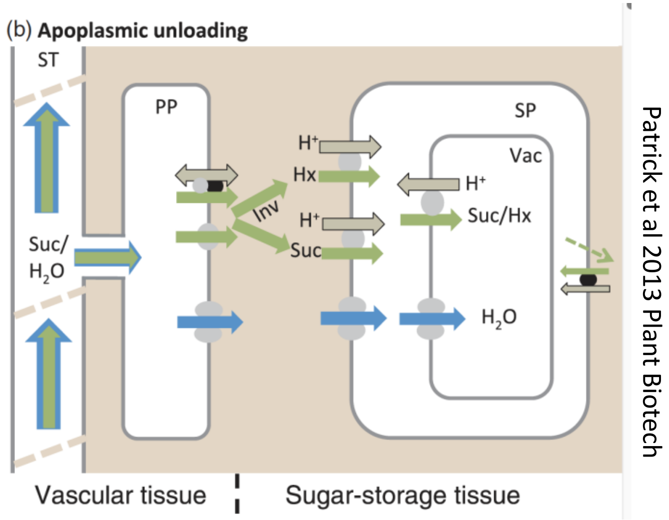
Stadler et al 2005 Plant J.



Example: root tip



Specialized transporters



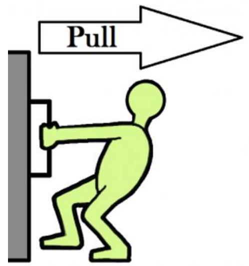
Example: fruits

SYMPLASMIC: convection + diffusion
depends on diffusion & bulk flow,
no energy is required
•slow, relative to energetic unloading

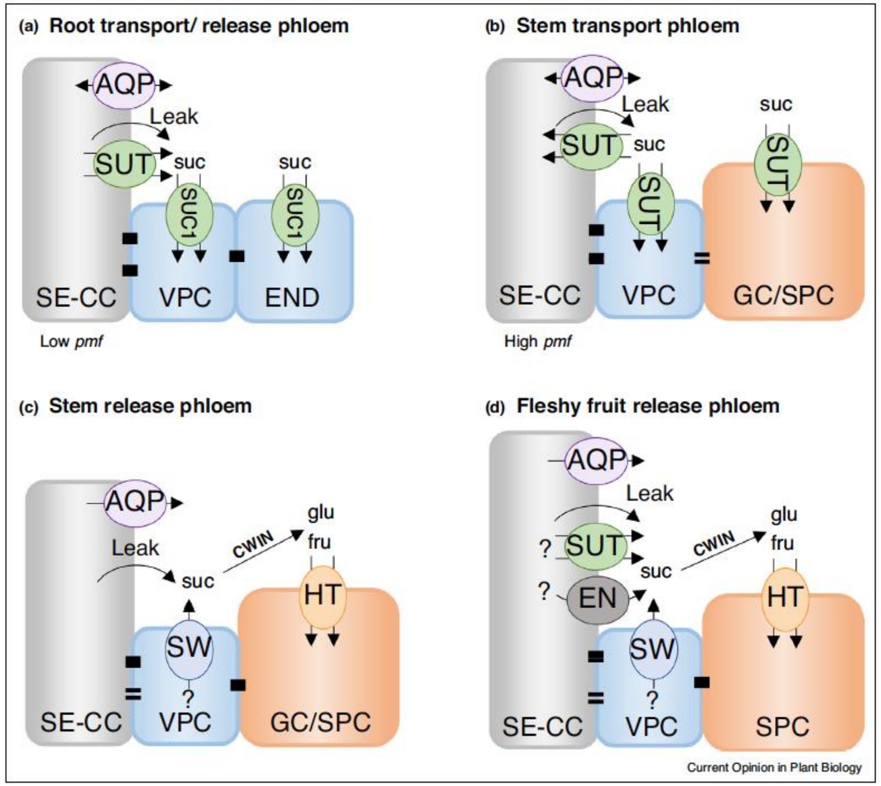
APOPLASMIC: energy-coupled process, against the concentration gradient
• high levels of soluble sugar. More potential for accumulating carbohydrates

→ Developmental switches between symplasmic and apoplasmic pathways

Phloem unloading and sink strength
2) each sink has its own strategy



-> Different phloem unloading pathways might be employed by different sink organs or tissues in one species.
-> Phloem unloading strategies in crop fruits have evolved in response to the requirements of carbohydrate distribution, yield, and quality



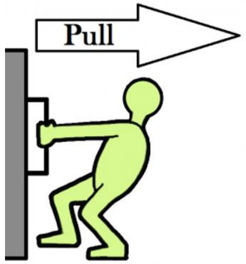
Closed PD
Open PD

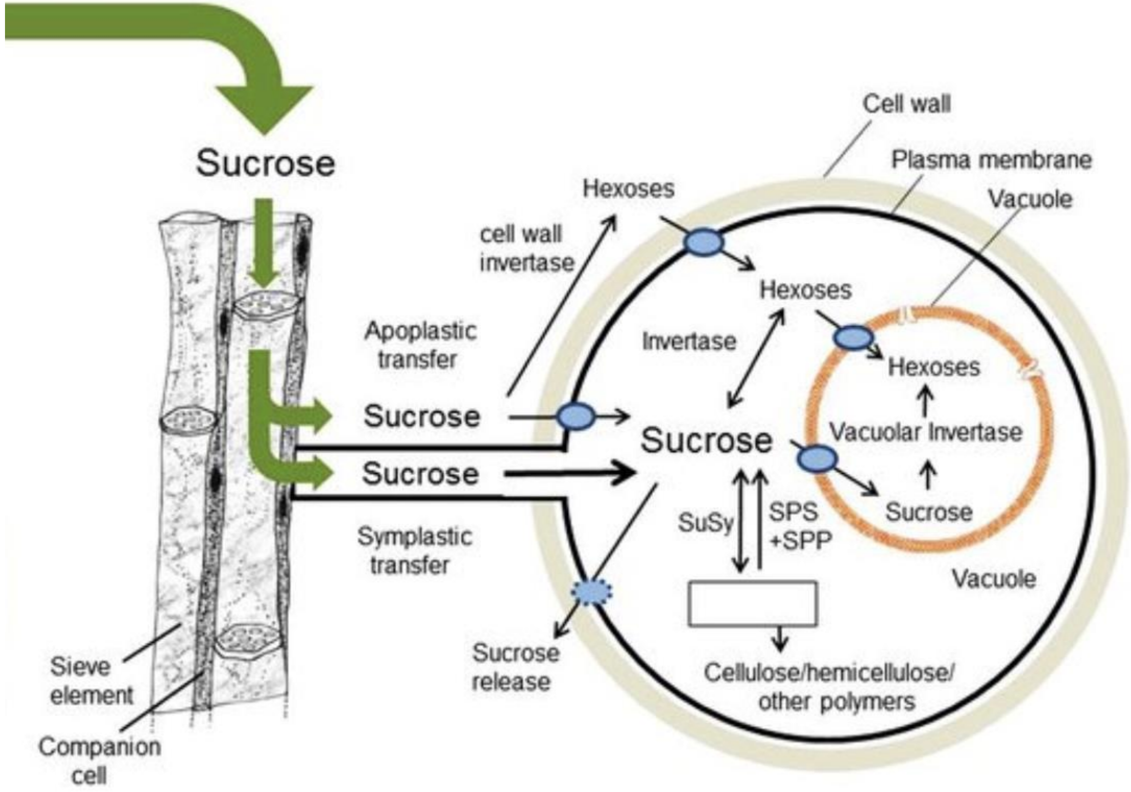
Milne, Grof, Patrick 2018 COPB

- proton motive force
- different transporters
- osmotic gradients & water availability
- metabolic activity of adjacent cells

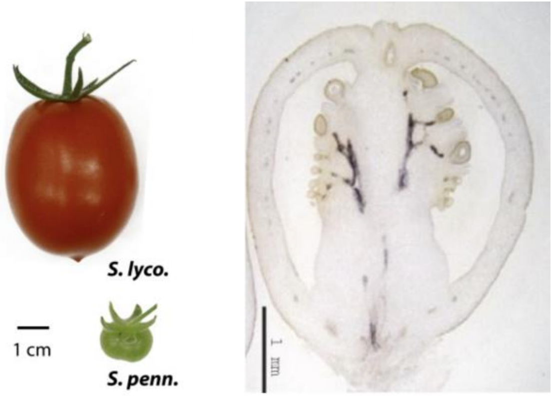
Examples
SWEET cucumber, fruit
SUC/SUT grape , fruit
HT (hexose transporters) grape fruit
TMT/PMT (tonoplast transporters) sweet sorgho, melon fruit
VST (tonoplast transport) watermelon fruit

Phloem unloading and sink strength
3) sugar use and partitioning





Wang et al., FIPS 2013

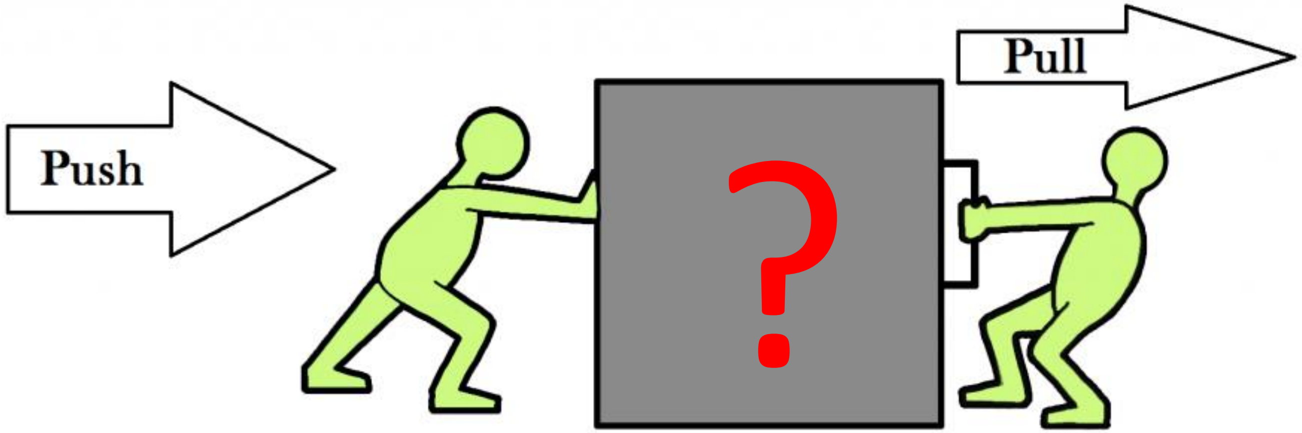


Ex: Brix9-2-5, QTL for sugar content in tomato fruit
-> increases sugars by as much as 25%
-> LIN5: *CwINV*,
mutation impairing enzyme kinetics and fruit sink strength

Fridman et al Science 2004

Source-to-sink relations

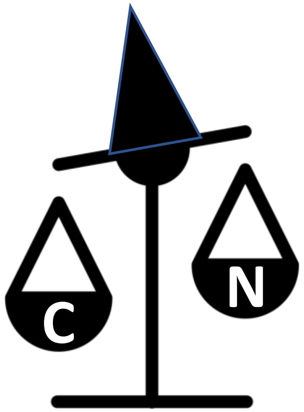
what about the other classes of metabolites?

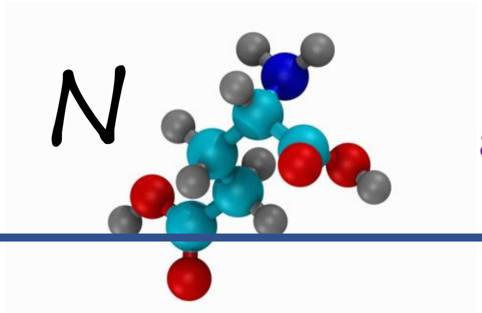


Phloem transport of N and amino acids

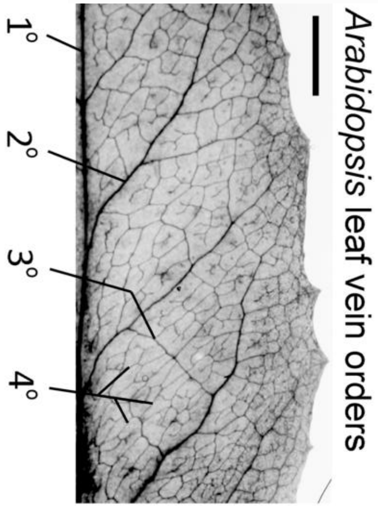
Coupling between C and N metabolism
-> Production of AA depends on C status

in the leaf : N remobilisation
forms of organic N transport
-> Gln, Glu, Asp, Asn, AA, Pro





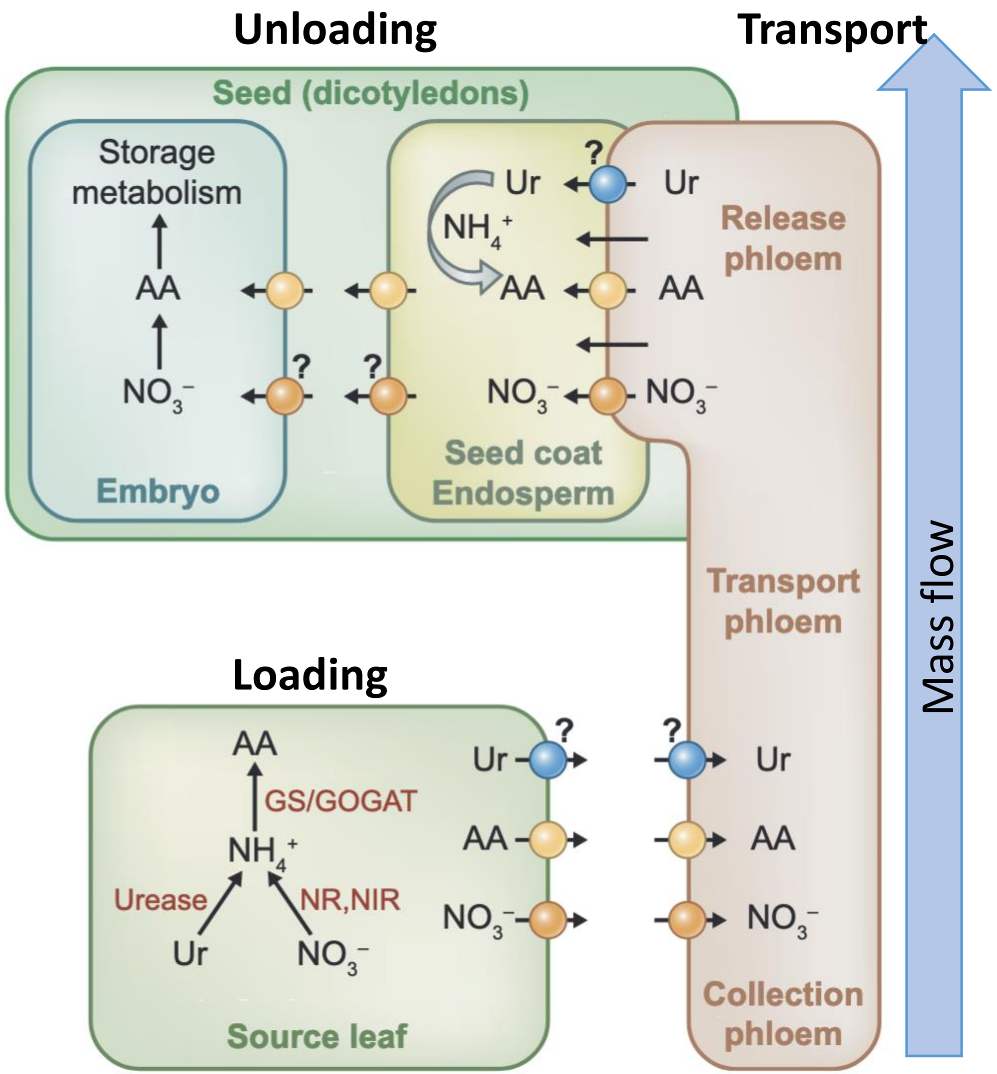
Different transporters of sugars and amino acids -> different regulations



- **Loading** of amino acids in all vein order
  -> via transporters
- **Transport**
  → convective , by mass flow
- **Unloading** via transporters

Open question: How is the export of sucrose co-ordinated with that of amino acids and ions?

N long distance transport: a variety of transporters



④ Seed vacuole

NRT2.7

③ Seed loading

NPF5.5?, UmamiT28, UmamiT29, CAT6, AAP8, AAP1

② Phloem unloading

NPF2.12/NRT1.6, UmamiT11, UmamiT14

① Leaf export/phloem loading

NPF2.13/NRT1.7, NPF1.2/NRT1.11, NPF1.1/NRT1.12, NRT2.4, NRT2.5, UmamiT18, AAP8

Tegeder, Masclaux-Daubresse 2018 New Phytol

Issues not yet addressed….

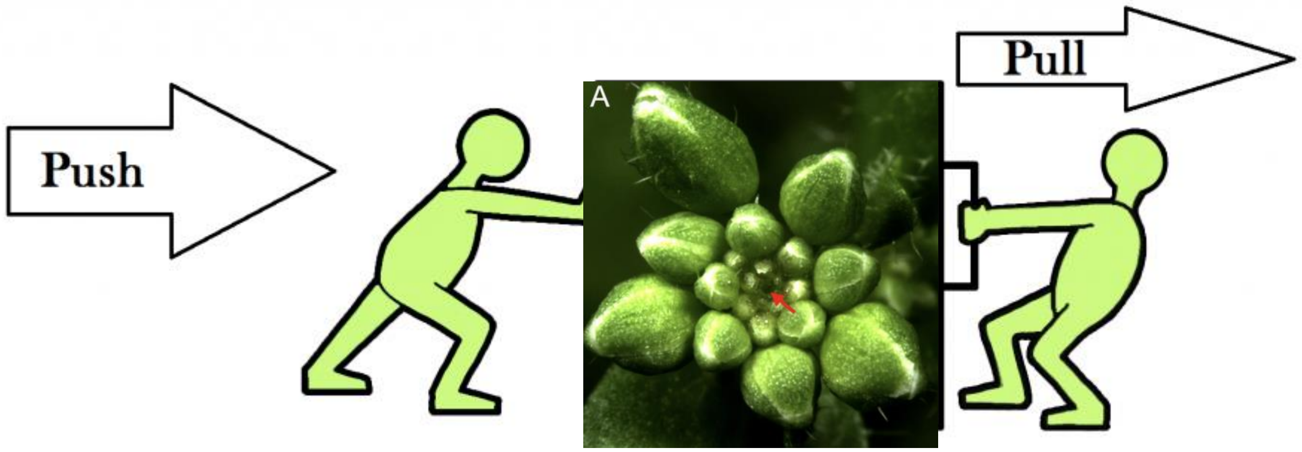
- What mechanisms and signals coordinate source and sink activity, and how do they respond to the environment?

- What determines the developmental switch between apoplasmic and symplasmic pathways observed in many sinks?

- What determines the number of sinks and how are sinks prioritized?

Source-to-sink relations

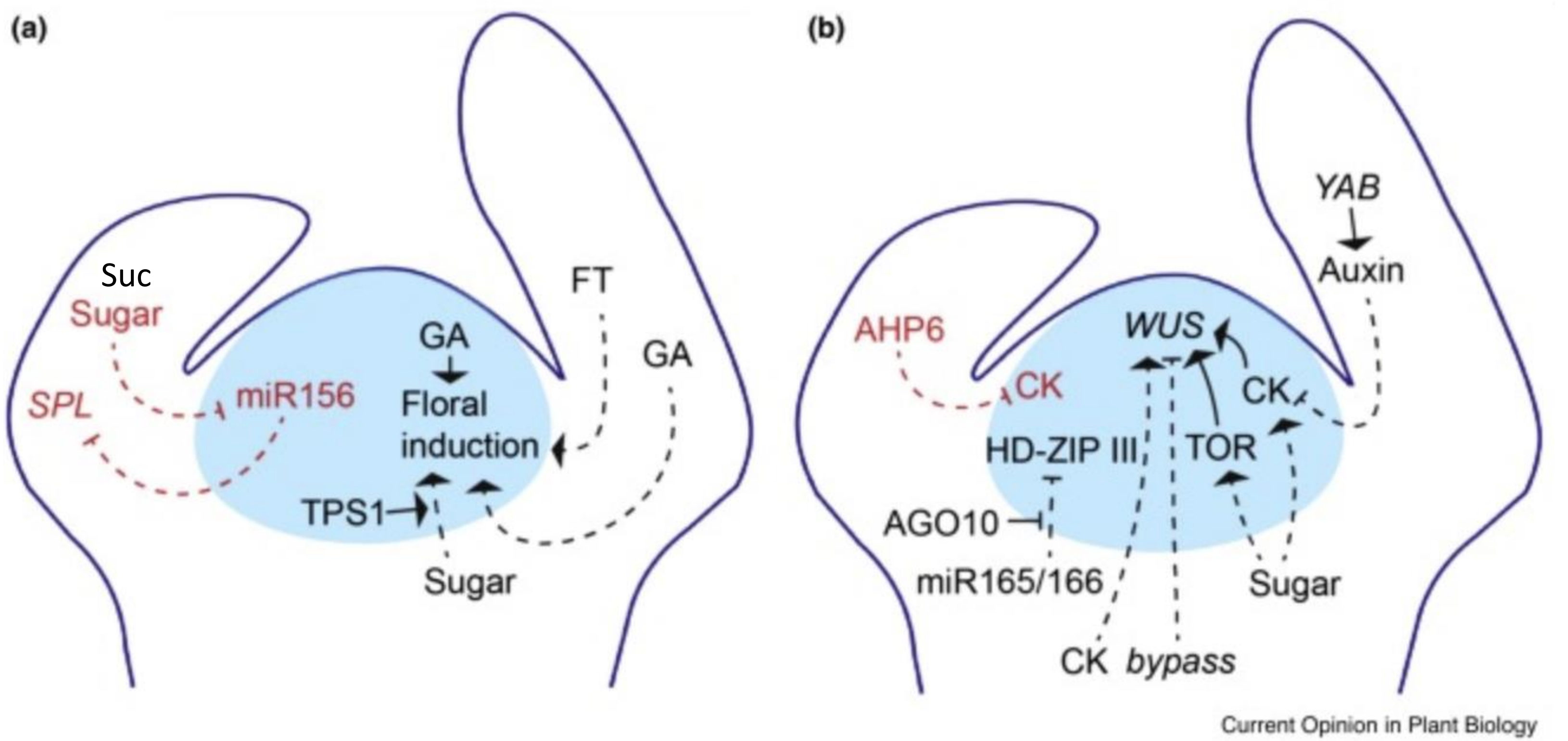
What about the SAM?



A important sink
What the factors control unloading?

Sugars as signal for SAM activity

-> Sugars , sucrose and glucose: signals at different stages of the SAM
-> Sugar availability : metabolite precursor or signal

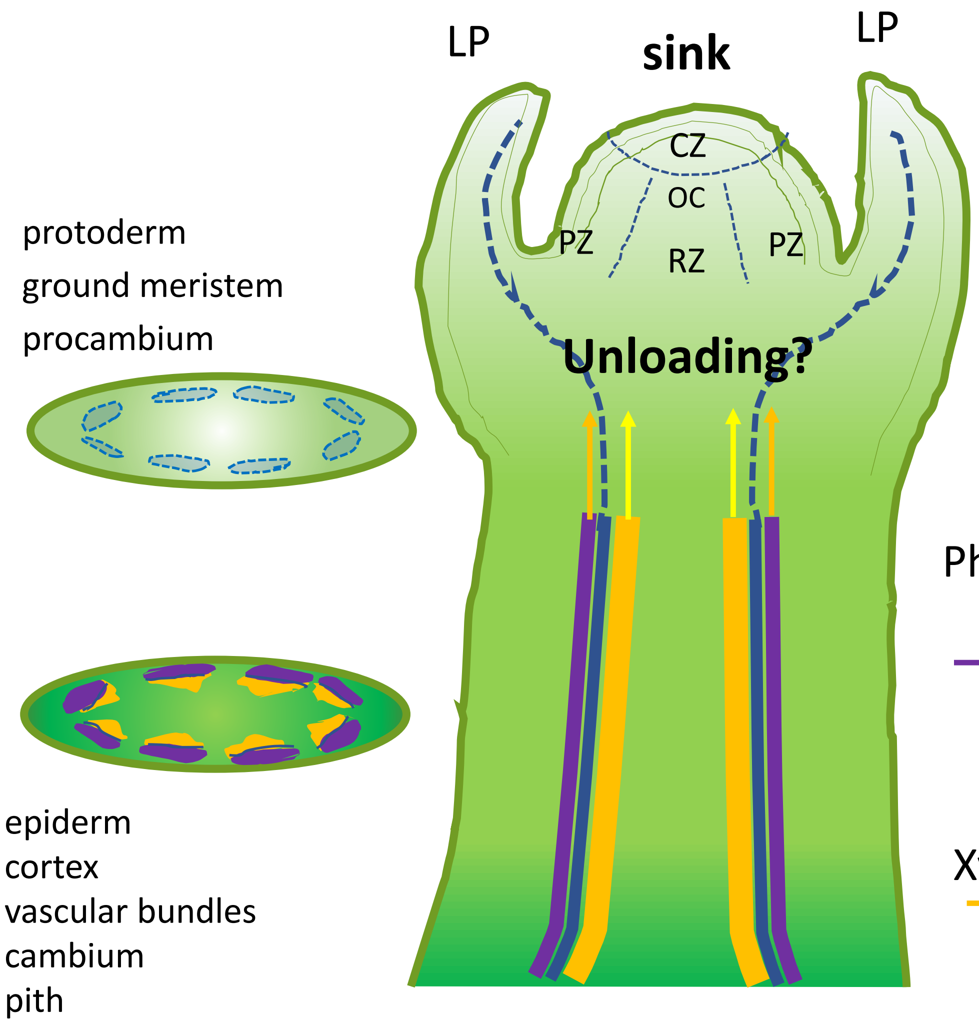


Fouracre & Poethig 2020 COPB

Mobile regulators in the shoot apex

(a) Vegetative phase change (red) and floral induction (black),
(b) Phyllotaxy (red) and meristem maintenance (black).

Unloading of sugars and nutrients in the SAM?



Phloem:

- Sucrose
- Polyols, raffinose (RFO)
- Organic acids
- Amino acids

Xylem:

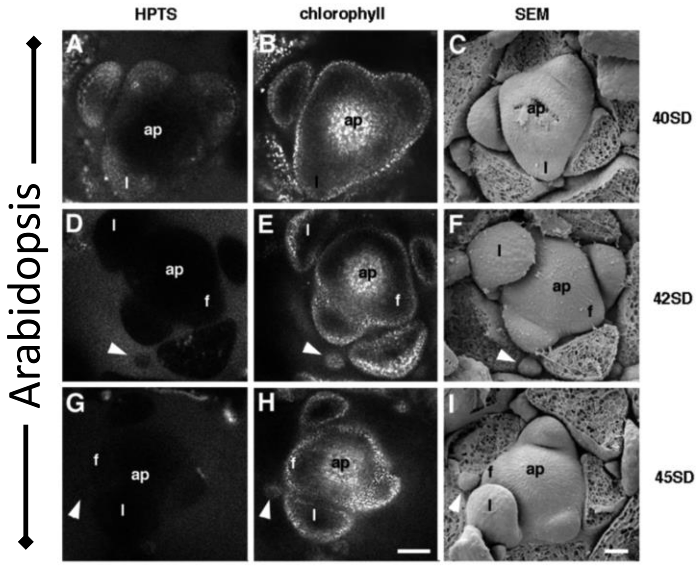
- Water
- Amino acids
- NO3-, NH4+
- PO4- ....

- Soluble sugars
- Starch
- CW polysaccharides
- Organic acids
- Amino acids
- Proteins
- Nucleic acids

Symplasmic isolation of the SAM

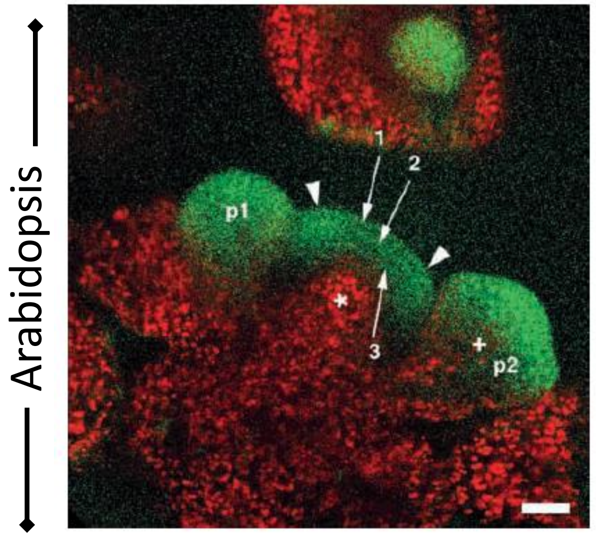
Vegetative phase, shoot apices are symplasmically isolated in the meristematic corpus
Shortly before and during the floral transition, the symplasmic domain extended throughout the whole shoot apex

Vegetative stage (SD)

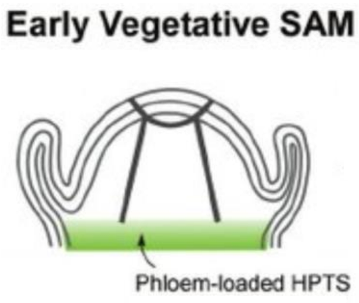


Gisel et al 1999 Development

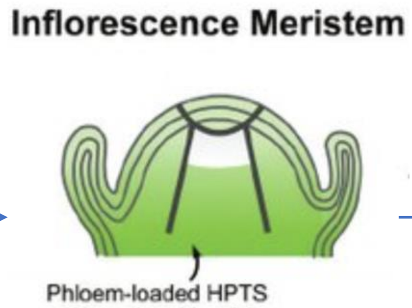
Reproductive stage (transfert SD to LD)

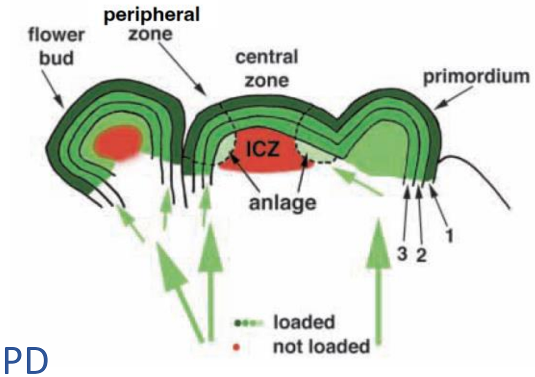


HPTS fluorescence



-> Efflux in the apoplasm
-> Uptake by active transport





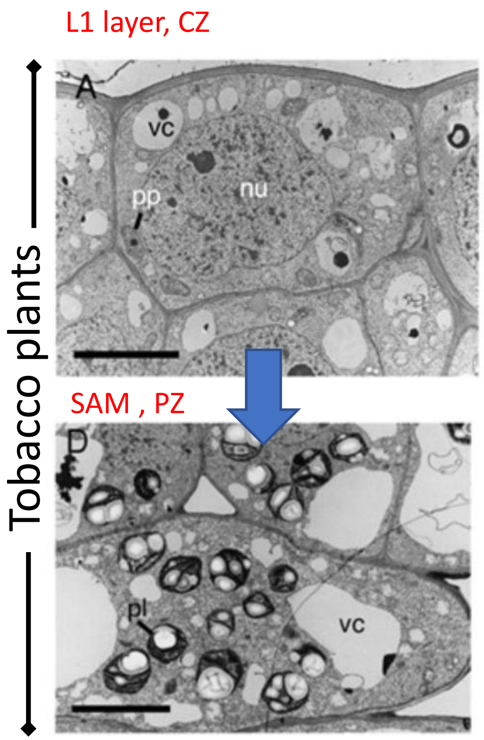
-> Diffusion by PD
-> Efflux/uptake in OC?

Carbohydrate metabolism in the SAM

- Spatial regulation in the vegetative apex

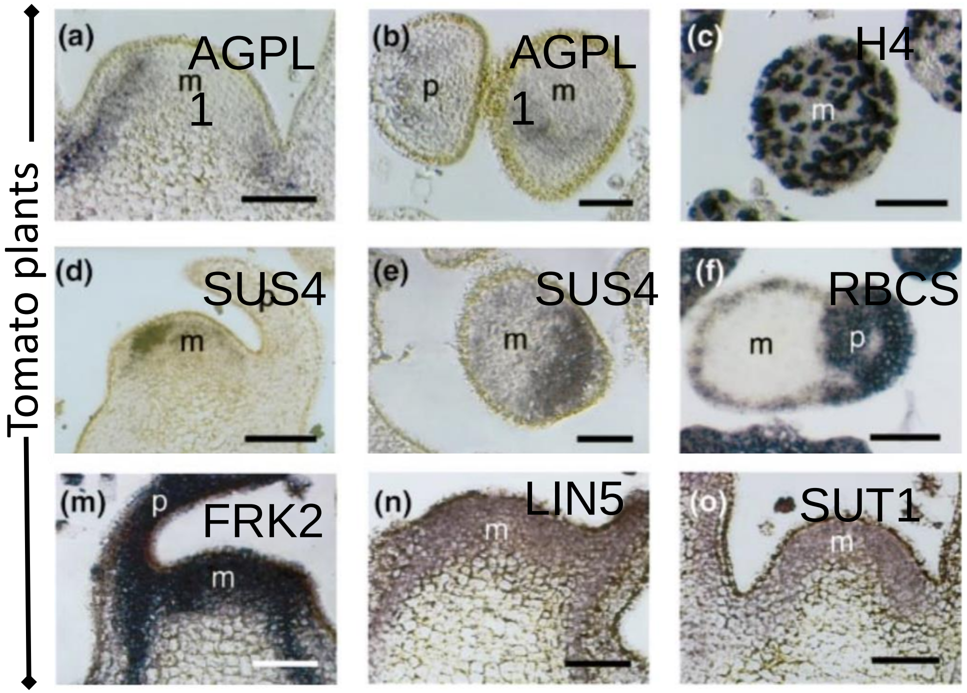
→ *Starch grains in the PZ of tobacco SAM*

→ *Starch grains in PZ of tomato SAM*



TEM of the vegetative SAM

Wyrzykowska et al. Plant Physiol 2006



HIS of the vegetative SAM

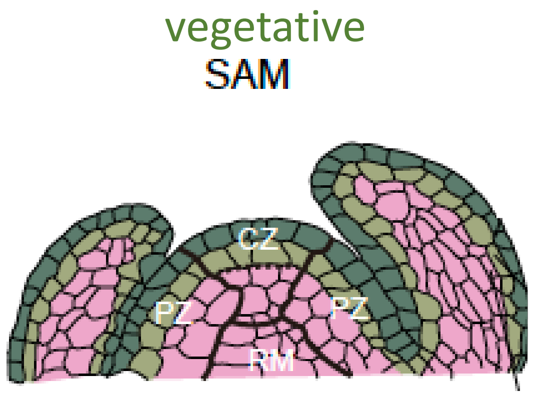
Pien et al. 2001 Plant J

ADPglucose pyrophosphorylase (AGPL1), sucrose synthase (SUS4)
-> dynamic accumulation of transcrips associated with primordium initiation and growth

Fructokinase (Frk2), invertase (Lin5), sucrose transporter (SUC2/SUT1):
-> uniform accumulation in the meristem

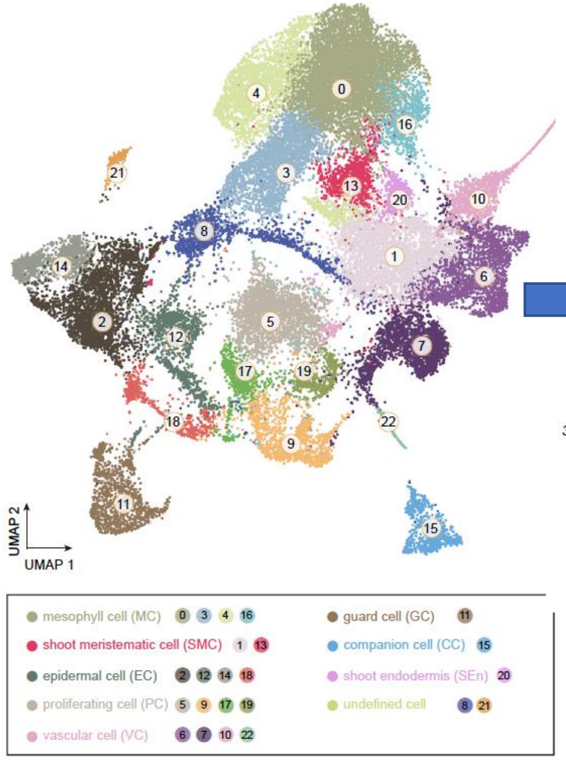
Sugar transport and metabolism markers in the SAM

-> Efflux in the apoplasm ?
-> Uptake by active transport ?

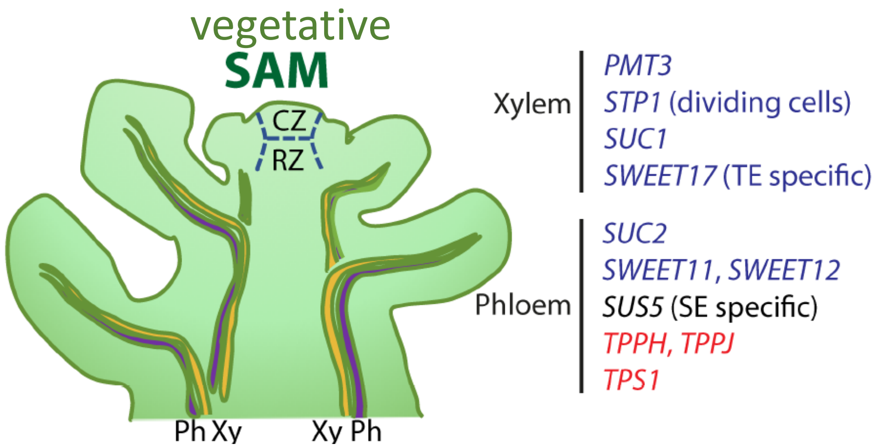


Single-cell compendium of the Arabidopsis vegetative shoot apex

Zhang et al 2021 Developmental Cell



Identification of cells with a vascular identity
- Transcripts for sugar transport or metabolism
- Transcripts for sugar signaling



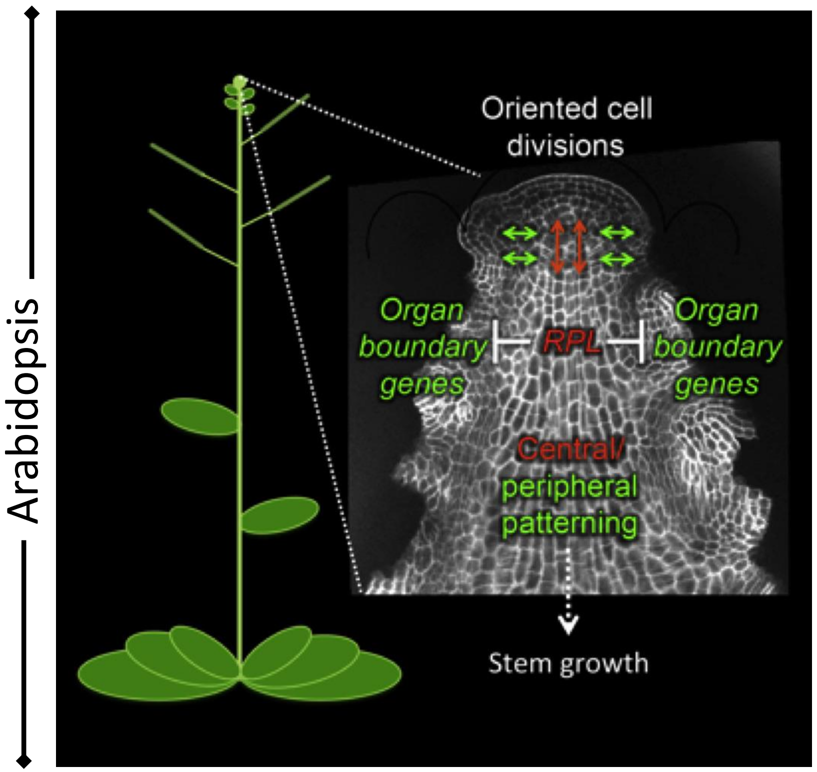
Dinant & Le Hir 2022 Physiologia plantarum

Sugar transport and metabolism markers in the rib zone of SAM

-> Diffusion by PD
-> efflux/uptake in OC?

RPL: REPLUMLESS transcriptional regulator
Expression in the *central and peripheral region of the RZ*

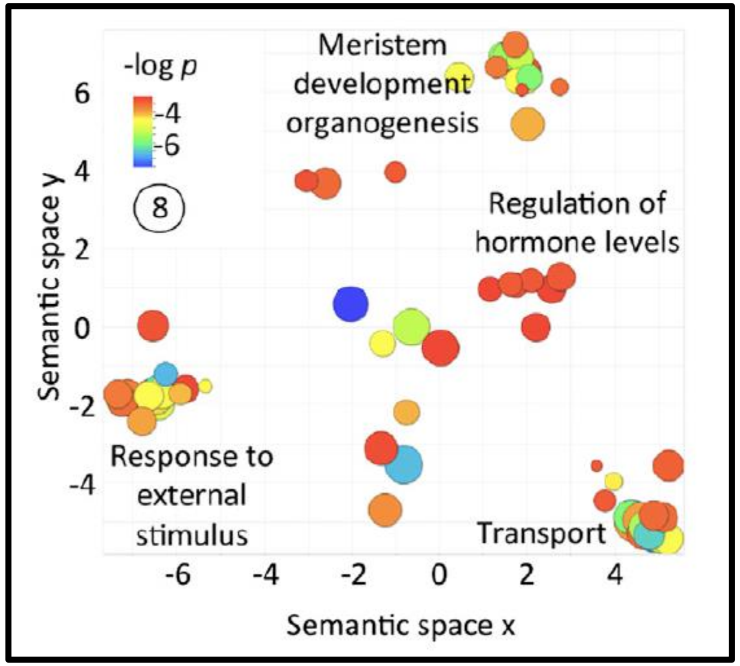
Inflorescence SAM



Bencivenga et al 2016 Developmental Cell

High-Confidence
RPL ChIP-Seq Targets

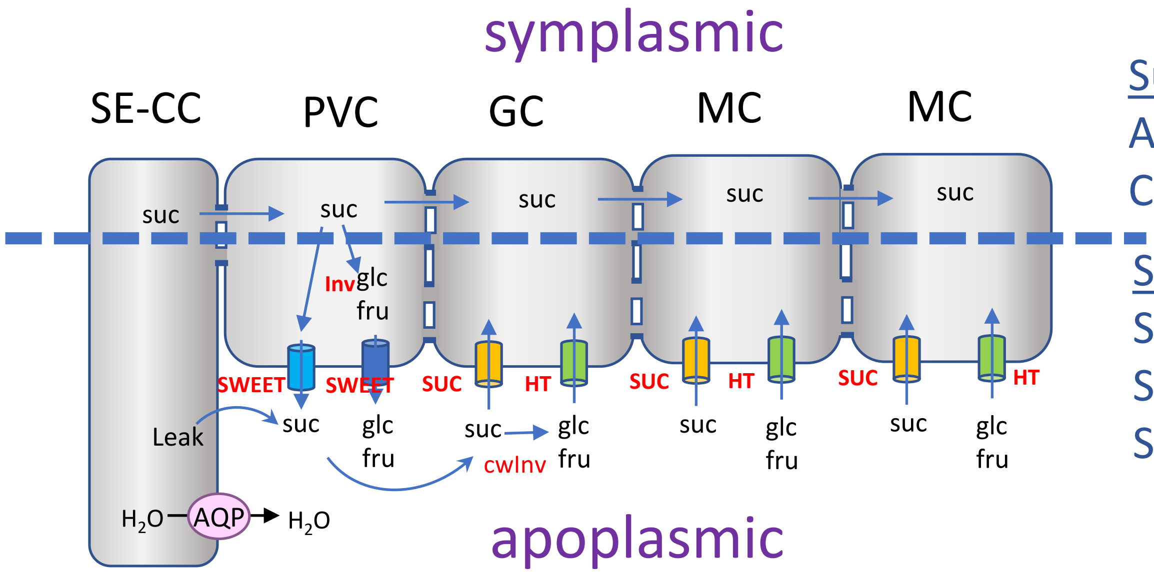
Transporters
SWEET1,14 (plasma mb)
SWEET2, 17 (tonoplast)
SUC1, SUT4 (plasma mb)
Metabolism
CwINV5 and V-Inv
SUS3
Other transporters
Aquaproin (PIP, TIP)
UMAMIT
AAP, PUP ...
Signaling
TPS1
TPP



inflorescence apices & ChIP-seq libraries
Enriched terms related to sugar transport

Unloading in the SAM ?

MC: meristem cells
GC: ground cells
PVC: perivascular cell
CC: companion cell
SE: sieve element



Sugar uptake/partitioning
AGPL, SUS4, FRK2
Cw-INV5, v-Inv, SUT1/SUC2,

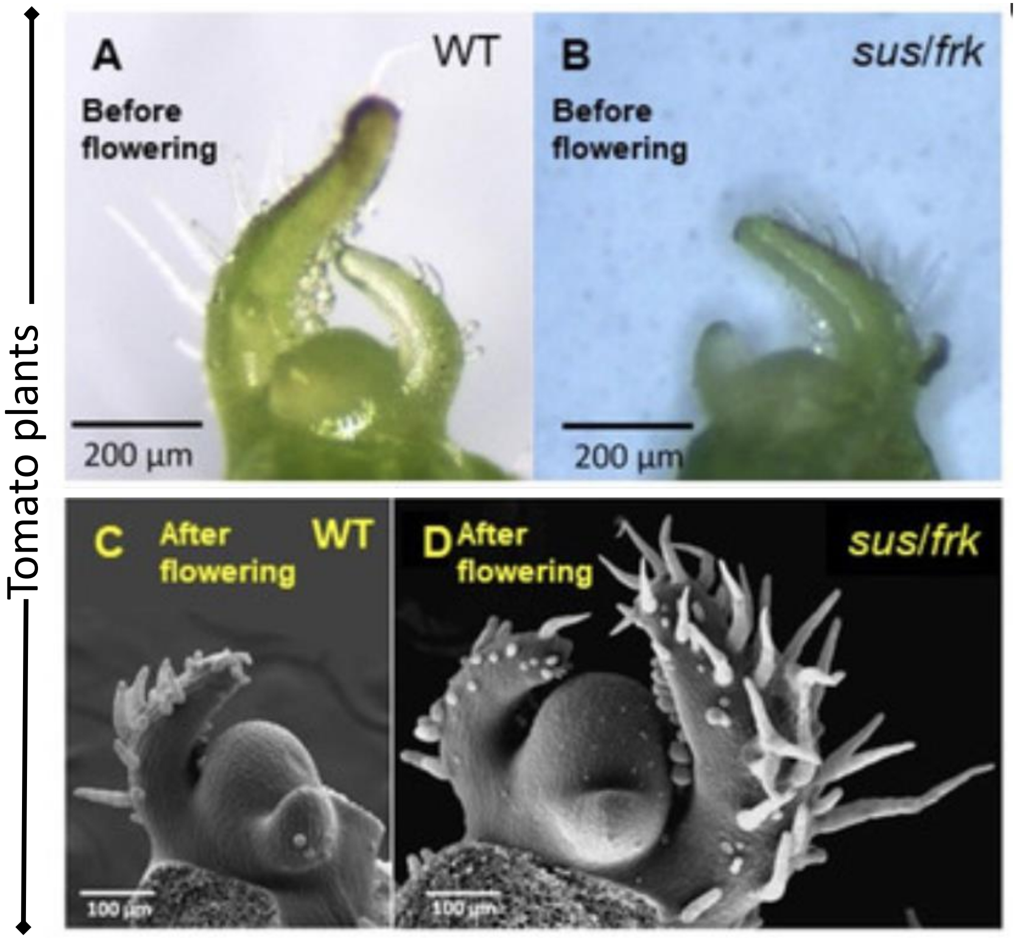
Sugar efflux or uptake
SWEET 2, 17, SUC4 (vac.)
SWEET 1 ,11, 12, 14 (PM)
STP1, SUC1 (PM)

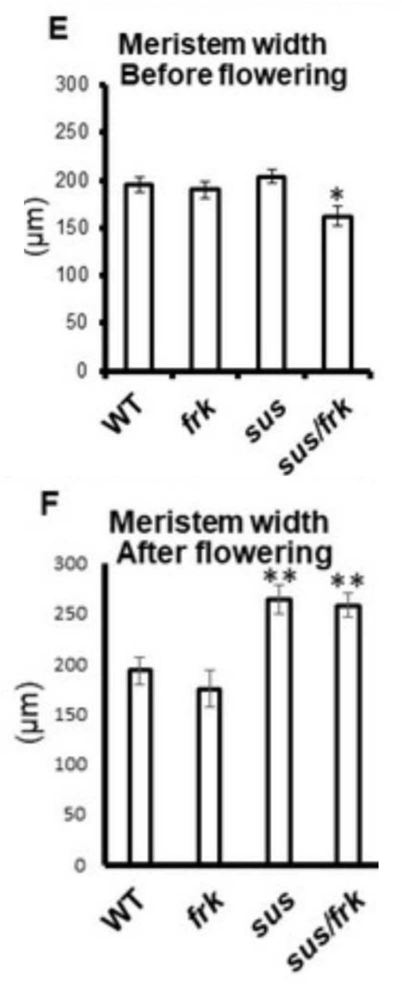
Heterotrophic cells → Autotrophic capacity

Sucrose synthase and fructokinase and SAM development

Tomato plants
Downregulation of *FRK2* and *SUSY* genes

- FRK2-antisense line
- SUS1,3,4-RNAi line





Goren et al., Plos One 2017 , Lugassi et al., Plants 2022

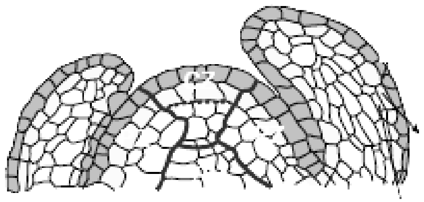
Sugar transport and SAM activity
Open questions

Regulation of Sink Strength at the different stages of development of the SAM ?

Switch apoplasmic to symplasmic

- Spatial and temporal regulation of
  - Sugar partitioning (vacuole, plastids, apoplasm)
  - Sugar availability ?

Pattern of expression in SAM?
- Sugar metabolism (CZ & PZ)
- Sugar transporters (RZ)



- Transient storage of soluble sugars (heterotrophic) ?
- Storage of starch (autotrophic cells) ?
- Cell expansion / cell division / maintenance ?
- Cell osmotic pressure ?

Is there storage of sugars in the SAM during the vegetative stage?
Can the SAM activity and maintainance be modified by changing the unloading or storage of sugars?
How to increase the sink strength of the SAM?

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